



Paradoxical effect of coating on natural guar gum blended carbomer matrix systems for the neurological depressive disorders

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Abstract

Oral extended release products offer potential advantages in patient compliance and therapeutic outcomes like sustained blood levels with attenuation of adverse effects. In neuropsychiatric disorders like depression, most of the formulations serve a marketing objective rather than a clinical objective. The present investigation was aimed to develop a once daily sustained release formulation for delivery of an acid-labile, water soluble antidepressant, duloxetine HCl. The formulation was pragmatically designed using blend of natural and synthetic polymeric biomaterials that it releases the drug at alkaline pH in a sustained manner. The basic intention was to develop a tablet formulation with hydrophilic matrix core, using blend of release retarding natural biodegradable polymers such as guar gum, carbopol 71G-NF (a synthetic carbomer) and C-Pharm[®] gel. Barrier coating using HPMC-E5 was given to retard the initial release followed by enteric coating with HPMC-AS to prevent exposure of drug in acidic milieu of the stomach. The formulation exhibited desired release pattern and was described best-fit by Hixon-Crowell model. Stability analysis under stress conditions up to one month displayed good reproducibility. The matrix tablets successfully decreased the symptoms of depression (significant decrease in immobility time) in a rat forced swimming model. Pharmacokinetic data of the formulation revealed ($t_{max} \sim 6$ h, $C_{max} \sim 1157.58$ ng/ml, mean $AUC_t \sim 11145.04$ ng*h/ml, and $K_a \sim 1.07h^{-1}$) good correlation in all animals.

Keywords: Matrix tablets; Duloxetine HCl; Sustained release; Enteric coating; Hixon-crowell model

Introduction

Depression is the 4th leading cause of disability worldwide and it will be the second leading cause of disability by 2020 second only to ischemic heart disease as reported by the WHO. A sharp increase in psychiatric disorders have been observed since the late 1950s, which is clear from the fact that 10% to 15% of prescriptions written in the U.S. are for medications intended to depression as a predominant disorder [1]. Depression is not a homogenous disorder, but a complex phenomenon which has many subtypes and

probably more than one etiology [2]. In one report by London school of Economics in 2006, "one in six of us would be diagnosed as having depression or chronic anxiety disorder" [3]. Depression is a kind of manifestation that can affect any age group. 3-6% children of age 4-16 years, who attended the child psychiatry out-patient clinics in Delhi, were found to be attacked by depression [4].

Most antidepressant drugs exert their actions on the metabolism of monoamine neurotransmitters and their

receptors, particularly norepinephrine and serotonin [5]. The major classes of drugs used to treat depression are the tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs, e.g., fluoxetine and sertraline), heterocyclics (e.g., bupropion), monoamine oxidase (MAO) inhibitors and a few other compounds such as venlafaxine and duloxetine, which inhibits specifically the reuptake of both serotonin and norepinephrine [6]. As on date, more than 80% of the therapeutic drugs are administered by the oral route and rated among high patient compliant dosage forms. Amongst these the immediate release tablets give the saw tooth pattern of plasma drug concentrations, which are further associated with adverse events at maximum concentration and loss of therapeutic effect at minimum concentration. This leads to intolerability or frequent dosing of many major categories of drugs including antidepressants. As incapability of the medication to deliver the drug at a proper time in a proper concentration to the required site is a major determinant of the therapeutic response achieved. Therefore these days drug reformulations are designed with the aim of reducing the pharmacokinetic inadequacies associated with the orally administered immediate release preparations. Modified dosage forms for oral drug-delivery systems often contain higher doses of a beneficial substance than do immediate-release preparations [7], and are typically designed to produce more uniform absorption of the beneficial substances delivered there from. Modified release formulations of antidepressant agents have the potential to improve tolerability by reducing adverse effects early in the course of therapy - a critical period of dramatic failure. [8] Duloxetine HCl is as a serotonin and norepinephrine reuptake inhibitor (SNRI) which inhibits dopamine reuptake and has no significant affinity for histaminergic, dopaminergic, cholinergic or adrenergic receptors. [9] Duloxetine HCl is an acid labile drug moiety. The development of a modified release formulation was preferred due to the various advantages offered which include the prevention of adverse events associated with the fluctuating plasma profile obtained with the immediate release formulations [7]. Hence, the aim of the study was to design i) a suitable delivery system which protects the drug from the acidic environment and allow the release of the drug for a prolonged period using various release retarding, film coating and enteric coating polymers.

The formulations were so designed that they do not release the drug in the acidic pH (stomach) consequently resulting in drug absorption after about two hours of the drug administration in sustained manner. ii) Characterization and evaluation for in vitro release, stability studies, pharmacodynamic and in vivo pharmacokinetic studies of the delivery system.

Materials and methods

Duloxetine Hydrochloride was a generous gift from Ranbaxy Laboratories Ltd. (Gurgaon, India). HPMC-E5, HPMC-AS and Guar gum were obtained as gift samples from Colorcon Asia Pvt. Ltd. (Mumbai, India) and Dabur Pvt. Ltd. (Ghaziabad, India), respectively. All the excipients and solvents used were of analytical grade.

Formulation of a matrix tablets

Matrix tablets of duloxetine HCl containing different amounts of polymer blend were prepared by direct compression along with magnesium stearate (lubricant) and talc (glidant). Tablets were made by first mixing all the excipients along with the drug thoroughly followed by sieving to obtain a uniform blend. The dry mix was blended uniformly with lubricant and glidant and then compressed into tablets on single punch machine (Modern Engg., New Delhi) using 8 mm. The core composition of matrix tablets containing different polymers in varying ratios has been shown in Table 1.

Coating of duloxetine HCl matrix tablets

Barrier coating of core tablets

The tablets were barrier coated at 2.5% w/w of coat weight using Gans-coater[®] (Gansons Limited, Thane, India) with an inlet air temperature of 35° C. The coating solution (3.0% w/v) containing HPMC E5 was prepared in mixture of ethanol and water (8:2) respectively. Di-butyl phthalate (15% w/w) was used as the plasticizer. A clear solution was obtained by continuous stirring on magnetic stirrer for sufficient period of time. The coating process was continued in order to obtain a required level of coat weight.

Enteric coating of core tablets

Batches obtained after 2.5% level barrier coating were further coated with enteric coating solution at different levels (3-13%), which were further used for release study. The enteric coating solution (3.0% w/v)

containing HPMC AS was prepared in mixture of ethanol and water (8:2). Di-butyl phthalate (15% w/w)

was used as the plasticizer. The solution was stirred on magnetic stirrer for sufficient period of time.

Table 1. Formulations containing various polymers ratios in the matrix core tablet

Formulation Code	Drug (mg/tablet)	Carbopol 71G NF (mg/tablet)	Guar gum (mg/tablet)	C-Pharm® gel (mg/tablet)	Magnesium stearate (mg/tablet)	Talc (mg/tablet)	MCC (mg/tablet)	Total weight
APL1	40	90	--	--	4.5	4.5	81.0	220
APL2	40	112.5	--	--	4.5	4.5	58.5	220
APL3	40	135	--	--	4.5	4.5	36.0	220
CPG1	40	90	--	83.25	2.25	4.5	--	220
GG1	40	90	2.25	81.0	2.25	4.5	--	220
GG2	40	90	4.5	78.75	2.25	4.5	--	220
GG3	40	90	9.0	74.25	2.25	4.5	--	220
C3G2	40	67.5	4.5	101.25	2.25	4.5	--	220

Evaluation of matrix tablets

Diameter and thickness measurement

All the batches were evaluated for diameter and thickness with the aid of a Vernier Caliper, in order to determine the uniformity in the size of batches.

Table 2. Comparative study of different drug release kinetic models [* Regression coefficient (R^2)]

Different Release Mechanisms for the Batch C3G2L1				Best Fit Model
Equation	Slope (n)	R^2		
Zero Order Release	$F = k_0 \cdot T$	-	0.905	-
First Order Release	$F = 100 (1 - e^{-kIt})$	-	0.938	-
Higuchi's Release	$F = kH\sqrt{t}$	-	0.925	-
Hixson-Crowell Release	$F = 100 [1 - (1 - K_{HC}t)^3]$	-	0.970	Hixson-Crowell
Korsmeyer-Peppas Release	$F = k_p t^n$	1.540	0.963	-

Hardness and friability

Hardness and friability of the tablets was determined using Monsanto hardness tester and Roche friability tester for all the batches respectively.

Table 3. Time of immobility of animals during FST in both the test sessions (n=6)

Group name	Mean time (in sec)		Standard Error Mean		Tukey's test	
	Control	Test	Control	Test	Control	test
Group A (2 h)	72	71.5	17.90	19.15	0.028	0.026
Group B (4 h)	60.83	41.33	11.21	6.05	1.74	3.22
Group C (6 h)	57.33	40.83	14.10	5.45	1.17	3.02
Group D (8 h)	61.33	44.66	10.19	11.43	1.64	3.39
Group E (12 h)	84.16	66.33	14.36	6.04	1.24	2.95
Group F (24 h)	82.50	62.00	14.94	8.13	1.37	2.52

Drug content

Ten tablets from each batch were weighed and powdered in a mortar and pestle. Powder equivalent to the drug in each tablet was weighed and dissolved in water to obtain a solution of 1 mg/ml. The solution was sonicated for 5 min then subsequently centrifuged for 10 min at 4000 rpm. After centrifugation, the supernatant was taken and then diluted with water. The drug content was then calculated by analyzing the sample using a UV-Visible spectrophotometer (Shimadzu -1601, USA) at λ_{max} of 291nm.

In-vitro release studies

The *in vitro* release profile of the drug in coated tablets was carried out according to USP- XXIII method for delayed release tablets (Method A). Dissolution studies were carried out using USP apparatus type-II i.e. paddle type at 50 rpm and at a temperature of 37 ± 0.5 °C. Initial studies were performed in 0.1N HCl (pH 1.2) for 2 hours followed by phosphate buffer (pH 6.8) up to 24 h. The pH was adjusted with the aid of 2N HCl or 2N NaOH. Samples were withdrawn at predetermined time intervals and replaced with fresh media. They were then analyzed using UV-spectrophotometer (Shimadzu-1601, USA) at λ_{max} of 291 nm.

HPLC analysis

The method for the drug estimation in plasma was validated by RP-HPLC system at λ_{max} - 232 nm (consisted of a LC-10 AT VP Shimadzu pump equipped with a SPD-10 AV P Shimadzu UV- visible detector) using BDS Hypersil C-8 reverse phase column (150 × 4.6 mm, S-5 μ) in a mobile phase Phosphate buffer (pH 2.5) and organic phase (methanol : tetrahydrofuran, 80:20) in the ratio of 55: 45 with flow rate of 1.0 ml/min at 40°C. The volume of injection used was 20 μ l.

Physiochemical compatibility studies

The physicochemical compatibility between drug and various excipients (Carbopol, C-Pharm Gel and guar gum) was investigated in order to avoid costly material wastage and time delays. The 1:1 physical mixtures of these excipients with the drug were subjected to DSC analysis. Because the gathering of real-time stability and compatibility data is incomplete in the early stages of development, stress testing and accelerated stability methods were also used for predicting ambient

condition interactions and rates of degradation for the drug candidate. Tablets were exposed to accelerated conditions ($40^\circ\text{C} \pm 2^\circ\text{C}/75\% \text{ RH} \pm 5\% \text{ RH}$) for different time periods (0, 15, 30, 45 days), and evaluated for the different physicochemical parameters viz. appearance, thickness, hardness, diameter, drug content, in vitro release studies and chemical drug degradation (using validated HPLC method). The results of DSC and HPLC were combined in order to assess the incompatibility and stability.

In-vivo pharmacodynamic studies

Animals were housed in accordance with the guidelines of Institutional Animal Care and Use Committee (IACUC) and were fed on standard normal pellet diet (Aashirwad Industries, Chandigarh) along with water *ad libitum*.

Rat forced swimming test (FST)

FST a behavioral phenomena, was performed using Spargue-Dawley rats weighing 190-225 g. Thirty six animals were selected (divided into six groups of six animals each) for the study. Swimming sessions were conducted by placing the rats in glass beaker (46 cm × 20 cm × 22 cm) containing water maintained at the height of 30 cm, so that rats could not support themselves by touching the bottom with their feet, at the temperature of 23-25°C. Three swimming sessions were conducted: an initial 15-min pretest followed 24 h later by a 5-min test; observations of this session were kept as control and then again followed 24 h later by a 5-min test [8]. Tablets were given orally to rats in a dose equivalent to 40 mg/kg of drug. Following the swimming sessions, the rats were removed from water, dried and then returned to their home cages [9]. The water in the beaker was changed after every trial. A time-sampling technique was employed to score the behavior during the test period and statistical analysis was applied on the data using Tukey's test to compare the values of the control group from experimental groups [10].

In- vivo pharmacokinetic studies

In order to determine the plasma level profiles of duloxetine HCl, tablets (in a dose equivalent to 40 mg/kg of drug) were administered orally in rats (n=5, 180-250 g) [11]. After oral dose, blood samples were withdrawn at intervals of 0, 2, 4, 6, 8, 12 and 24 h.

Blood samples were collected in micro-centrifuge tubes containing heparin and were centrifuged (5000 rpm for 5 min). The separated plasma was kept at -20°C if not analyzed immediately. The drug extracted from the plasma using acetonitrile (1 ml in 500 μl of plasma) was subjected to HPLC analysis. All the pharmacokinetic parameters were calculated for individual animals using Jandel software (Sigma stat[®] 2.0). Then plasma profile of all the animals were plotted as mean \pm SD. Moreover, SPSS[®] (version 16.0) curve-fitting program using least square technique was used to evaluate the correlation between the mean plasma concentration ($\text{AUC}_{0-24\text{h}}$) against different time intervals and compare their relative goodness of fit for models where a single dependent variable is predicted by a single independent variable or by a time variable as shown in Fig. 8.

Results and discussion

Tablets batches were tested for uniformity of weight as per I.P. and were found to be within specified limits. Other parameters hardness, diameter, thickness, friability and drug content of various formulations were found to be in range of 12.0 - 16.5 kg/cm², 8.02 ± 0.02 - 8.46 ± 0.02 (mm \pm S.D), 4.28 ± 0.03 - 5.0 ± 0.02 (mm \pm S.D), 0.02% - 0.14% and 97.98% - 100.72% respectively.

Preliminary studies were carried out using various core tablet matrix systems for modified delivery of duloxetine HCl. The effect of fillers like MCC and C-Pharm[®] gel was evaluated for the release characteristics of the drug. Three batches containing duloxetine HCl and Carbopol 71G NF (40%-APL1, 50%-APL2, and 60%-APL3) were prepared by direct compression using microcrystalline cellulose (MCC) as the filler (Table 1). In vitro release profile showed burst release after 8 h for Batch APL1 (Figure 1) which might be due to low concentration of polymer and decrease in interactions between the carbopol polymer linkages by MCC [12-13]. However, in case of batches APL2 and APL3, the complete drug could not be released after 24 h may be due to higher content of carbopol thus overcoming the disintegrating power of MCC [14].

Further studies were performed by replacing MCC with C-Pharm[®] gel to provide complete release accompanied with the desired initial release retardation. Batch containing duloxetine HCl and

Carbopol (90 mg) with 37.08% of C-Pharm[®] gel (CPG1) was prepared and exhibited initial drug release of 8.88% in 2 h and 100.81% in 24 h.

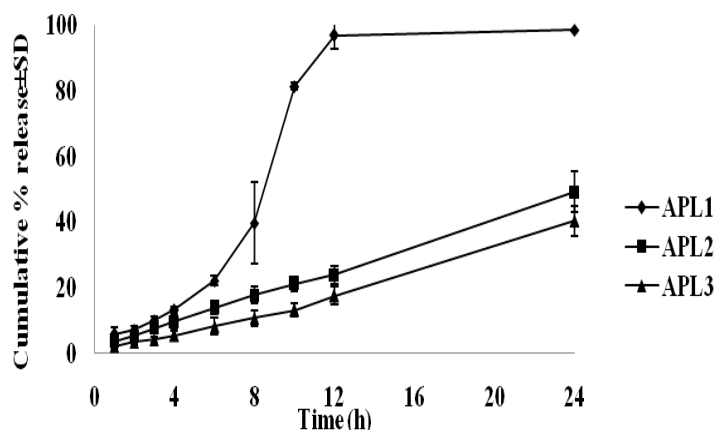


Figure 1. Plot of cumulative percent release of duloxetine HCl from matrices containing Carbopol 71G NF and MCC (APL1, APL2 and APL3) mean \pm SD; n=3.

Matrix tablets containing carbopol and guar gum using C-Pharm[®] gel as filler

Further, investigation was designed with guar gum as a dry binder at low concentration and C-Pharm[®] gel as filler which offered additional benefits such as ease of formulation, complete release accompanied with the desired initial release retardation.

Three batches (GG-1, GG-2, and GG-3) containing duloxetine HCl, Carbopol 71G-NF (90 mg) with different amounts of guar gum 1%, 2% and 4% respectively and one batch with 67.5 mg carbopol 71G NF and 2% guar gum (C3G2) were prepared by direct compression using C-Pharm[®] gel as the filler. In-vitro dissolution profile of Batch GG1 showed a cumulative percent release of 7.13% in first 2 h; 19.70% in 6 h and 99.77% in 24 h. Tablets of batch GG2 showed a cumulative percent release of 4.55% in first 2 h; 11.92% in 6 h and 77.43% in 24 h (Figure 2). In case of batch GG3, 5.50% drug release in first 2h; 10.94% in 6 h and 41.16% in 24 h. Tablets of C3G2 exhibited cumulative percent release of 8.11% in 2h; 40.85% release in 6 h and 96.29% release in 24 h. The concentration of Carbopol remained same in all three batches, however with increasing the amount of guar gum (GG-1, GG-2, and GG-3); there was decrease in the drug release from the respective formulations.

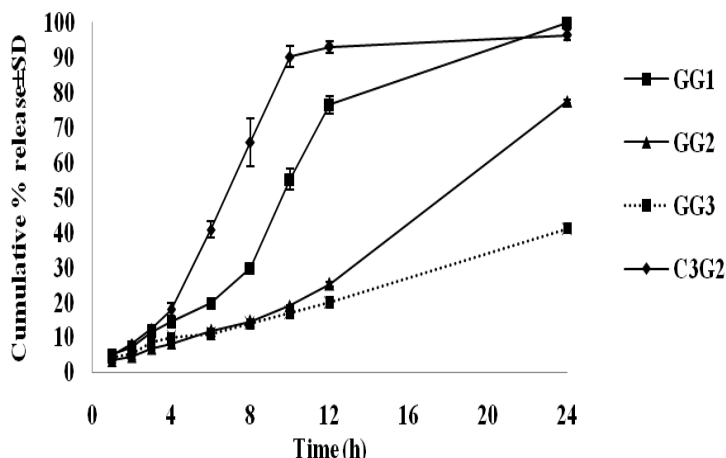


Figure 2. Plot of cumulative percent release of duloxetine HCl from matrices containing Carbopol, Guar gum and C-Pharm® gel (GG-1, GG-2, GG-3 and C3G2) mean \pm SD; n=3.

High initial retardation was achieved in all batches GG1, GG2 and GG3 but no complete release of drug was found to occur at 24 h except batch GG1 (Figure 2). However, in case of batch C3G2 the complete drug was released in sustained manner. This formulation was enteric coated in order to prevent drug degradation in acidic environment of the stomach. Initially, a barrier coat of HPMC-E5 which would act as a seal coat was given then eventually a second coating with enteric coated polymer was provided as the enteric coating material on dissolution creates an acidic environment which may lead to degradation of the drug that can be prevented by the barrier layer.

The drug release profile of the seal coated tablets (coated with HPMC-E5, 2.5% coat weight) of final batch (C3G2FC) revealed that seal coating itself was not retarding the drug release to a great extent (Figure 3). This is done just to prevent interaction between enteric coated polymer and drug in the matrix.

Enteric coating of batches GG1 and C3G2

In the subsequent study, the purpose of the enteric coat was to delay the release of duloxetine HCl until it reached small intestine because drug is degradable in acidic stomach. HPMCAS (Hydroxy propylmethylcellulose acetate succinate) was used for enteric coating. Tablets were coated with enteric polymer to different level to tailor the drug release. Cumulative percent release of drug after 24 h from

both the uncoated (GG1) and coated batches (GG1L1, GG1L2 and GG1L3), are shown in Figure 4.

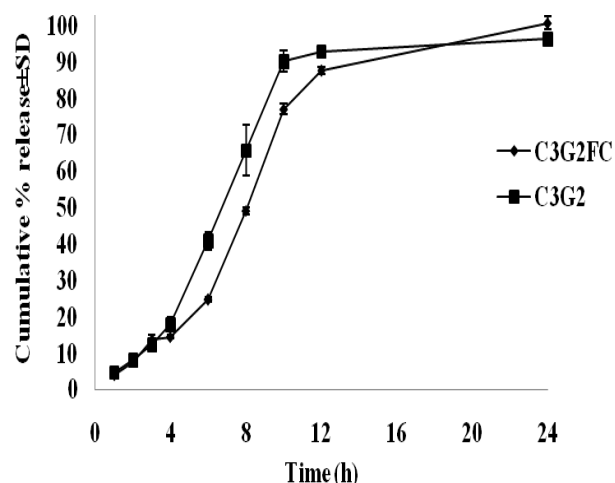


Figure 3. Plot of cumulative percent release of duloxetine HCl from Barrier Coated Tablets.

The tablets of batch GG1 containing 40% carbopol 71G NF and 1% guar gum, showed a cumulative percent release of 7.13% in first 2 h and was 99.77% in 24 h as depicted in Figure 2. In order to prevent initial drug release the tablets were enteric coated with HPMCAS at three different levels of 3%, 8% and 13% for batches GG1L1, GG1L2 and GG1L3 respectively. Batch GG1L1 showed a release of 1.22% after 2 h and 97.33% at the completion of 24 h was observed (Figure 4).

A negligible amount of the drug was released till 2 h and also drug was released in a sustained manner with complete release of the drug at 24 h. As coat weight of 8% (GG1L2) drug release was negligible at 2 h but release of the drug was much sustained although complete drug was released at final hours. In case of batch GG1L3 with highest coat weight of 13% the drug release was highly retarded and the same time tablet failed to release the complete drug. Hence, these two batches were not suitable as sustained release tablet.

Batch (C3G2) was also enteric coated to develop sustained release of duloxetine HCl using HPMCAS. The batch C3G2 (uncoated) showed a cumulative percent release of 8.11% in first 2 h and was 96.29% in 24 h (Figure 2). In order to prevent drug release in stomach, the batch was enteric coated with HPMCAS at four different levels 2%, 3%, 4.4% and 6.8% for batches C3G2L1, C3G2L2, C3G2L3 and C3G2L4.

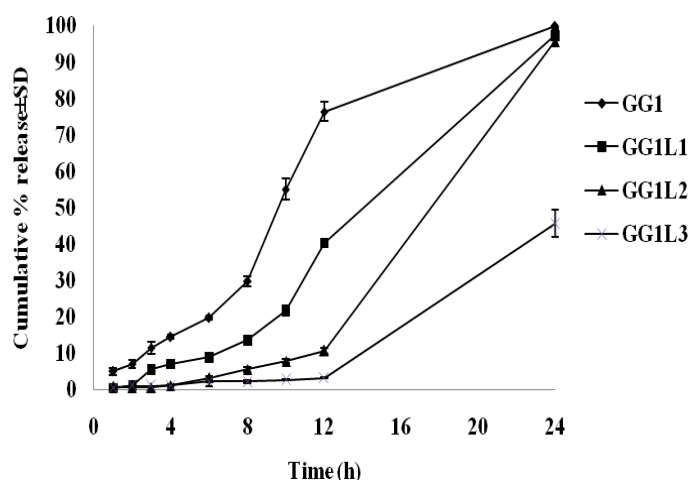


Figure 4. Plot of cumulative percent release of duloxetine HCl from matrices containing carbopol, Guar gum and C-Pharm® gel coated at various levels with HPMCAS (3% w/w) mean \pm SD; n=3.

Batch C3G2L1 showed a release of 2.24% after 2 h and 98.53% at the completion of 24 h. A negligible amount of the drug was released till 2 h and also drug was released in a sustained manner with complete release of the drug at 24 h (Figure 5). At coat weight of 3% drug release was negligible at 2 h but release of the drug was more sustained although complete drug was released at final hours. In case of batch C3G2L3 with coat weight 4.4%, the drug release was much more sustained which was not desirable. On further increase the coat weight to 6.8% for batch C3G2L4, the release was highly sustained. Hence these three batches C3G2L2, C3G2L3 and C3G2L4 were not found to be suitable as sustained release formulation as none of these were able to provide a sustained release of the chosen candidate in the later hours. Batch C3G2L1 was observed to be the most suitable because it prevented drug release in the stomach and further provided sustained release to a sufficient extent i.e. it provided a uniform rate of drug release in the lower segment of the GIT. Hence, the batch C3G2L1 was further subjected to animal studies. The compatibility between duloxetine HCl and the various excipients (Carbopol 71G NF, C-Pharm® gel and guar gum) used in preparation of the tablets (Batch C3G2L1) was tested using thermal (DSC) and well developed analytical RP-HPLC method. The DSC curve of the pure drug exhibited a sharp peak at 164.55°C, corresponding to its melting point (Figure

6a). The DSC curve of the 1:1 mixtures of the excipients and the drug showed sharp drug peak indicative of its melting point (Figure 6b). This indicates the absence of physicochemical interaction of duloxetine HCl with different excipients used in the study.

Further, HPLC-based accelerated stability studies were conducted on the tablets by kept in high-density polyethylene bottles sealed with parafilm, at 40°C/75% relative humidity (Table 5). The studies indicated the complete stability of the formulation even after exposure to accelerated stability conditions.

Also, the kinetics of the release behavior was assessed for batch C3G2L1 (Carbopol-Guar gum-HPMCAS coated) by mathematical modeling. The formulation complied with higher correlation with Hixson-Crowell's cube root of time equation release mechanism as depicted in Table 2.

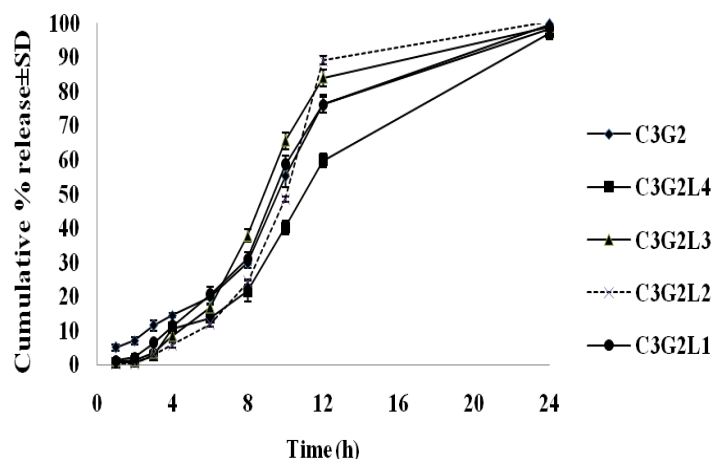


Figure 5. Plot of cumulative percent release of duloxetine HCl from matrices containing Carbopol (30%), Guar gum (2%) and C-Pharm® gel coated at various levels with HPMCAS (3% w/w) mean \pm SD; n=3.

Pharmacodynamic studies using rat forced swimming test

The data obtained from the six groups (Table 3) showed that there was no statistical significant difference ($P < 0.05$) in the behavior of the animals of Group A (dose was given 2 h before the test session) whereas statistical difference ($P < 0.05$) in the immobility behavior was observed in case of Group B,

Group C, Group D, Group E and Group F animals (dosing was done 4 h, 6 h, 8 h, 12 h and 24 h respectively before the test session) (Figure 7). From the results it was concluded that the release of the drug from the formulation was after 2 h and was released up

to 24 h after the administration. Thus, the investigation suggested the effectiveness of duloxetine (40 mg/kg in the form of matrix system) in reducing FST-induced depressive behavior.

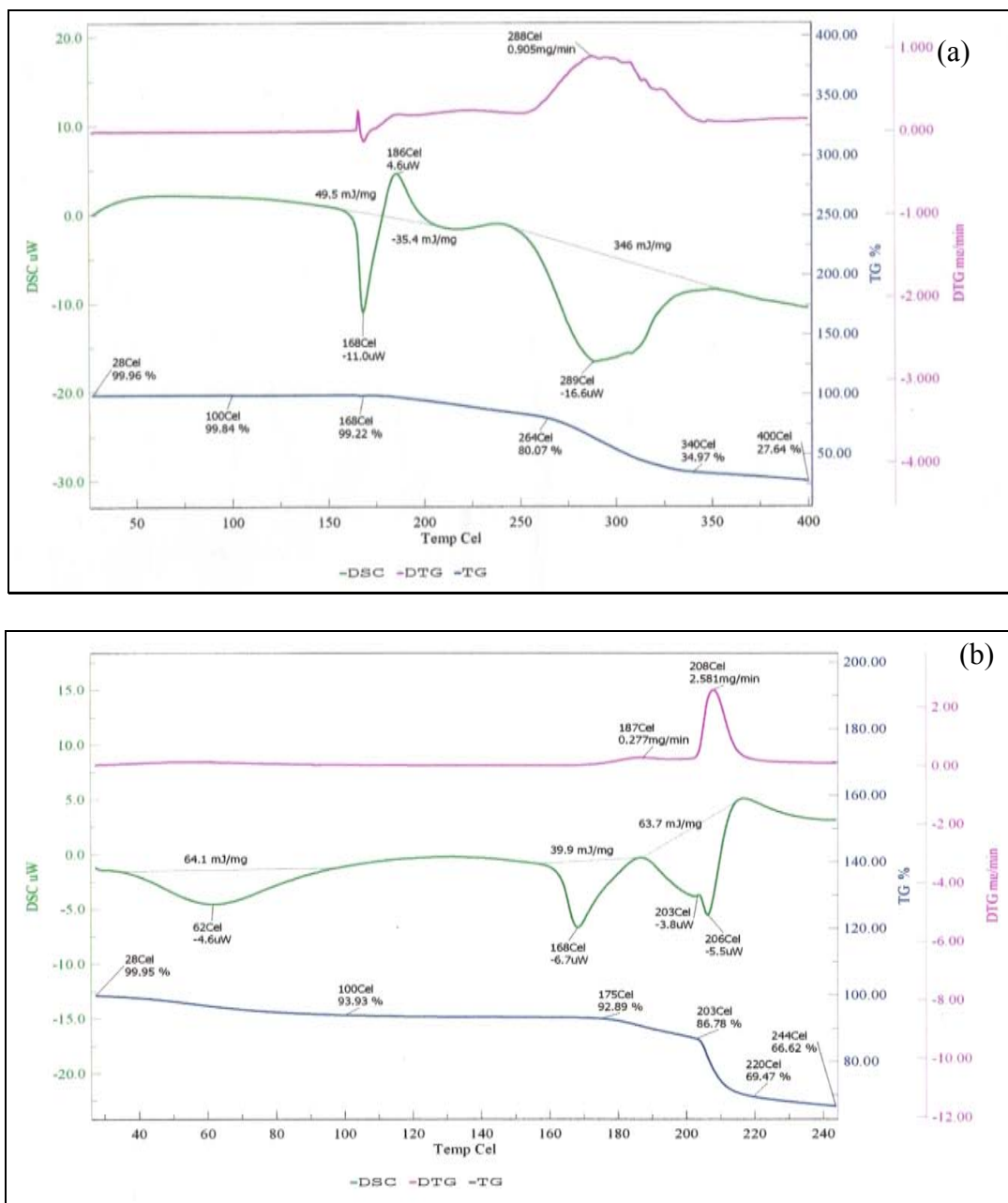


Figure 6. DSC Thermograms of the physical mixture of the drug with the tablet excipients (1:1) (a) Duloxetine HCl: Carbopol 71 G NF (b) Duloxetine HCl: guar gum.

In- vivo pharmacokinetic study

The sustained release performance of the developed dosage form was evaluated by in-vivo pharmacokinetic studies in the rats. Various pharmacokinetic parameters such as C_{max} , T_{max} , AUC_t , and K_a were obtained after drug plasma level studies. After administration of duloxetine (40 mg/kg), the drug plasma concentrations were monitored for 24 h and summarized in Table 4. The mean pharmacokinetic parameters (n=5) for batch C3G2L1 (Table 4) inferred that maximum therapeutic effect of the drug was observed within 6 h of administration and no drug release occurred in the initial 2 h which indicate the effectiveness of the enteric coat applied on the formulation. All the pharmacokinetic parameters obtained i.e. C_{max} , T_{max} , AUC , and K_a indicated good correlation in all the animals. The mean plasma concentration (AUC_{0-24h}) time profile was subjected to curve fitting models using least square method. The data for mean plasma concentration vs time showed best fit in cubic model with correlation ($r^2 = 0.556$, ($p < 0.05$)) for batch C3G2L1 at different time intervals.

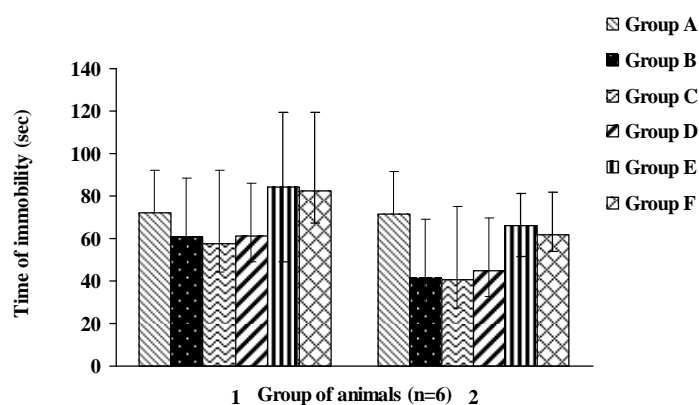


Figure 7. Comparison of the time of immobility in different groups during the two test sessions of FST (1-Control, 2-Test).

Stability analysis

Stability studies were performed at 40°C/75 % RH for one month to assess the stability of the Batch C3G2L1. After storage the tablets were subjected to assay of drug content and dissolution by a validated RP-HPLC method at specified time intervals of 0, 15 and 30 days respectively. No changes in the physical appearance and drug content were noted (Table 5). The statistical comparison (ANOVA followed by Tukey's test) using

Jandel software (Sigma stat[®] 2.0) showed that there was no significant difference in the amount of duloxetine HCl released at the end of 24 h dissolution study in all the stability samples.

Table 4. In-vivo pharmacokinetic parameters of tablet formulation in rats

Subject Rats (n=5)	T_{max} (h)	C_{max} (ng/ml)	$AUC_{0-\infty}$ (ng*h/ml)	AUC_t (ng*h/ml)	K_a
1	6	870.42	15543.21	8628.92	0.92
2	6	1157.58	61026.81	11184.81	1.18
3	6	1603.58	33048.27	13269.27	1.00
4	6	1137.79	41241.07	11651.57	1.17
5	6	1021.37	18777.36	10990.61	1.07
Mean	6	1158.15	33927.34	11145.04	1.07
SD	0.98	274.01	18412.92	1667.72	0.11

Selection of a proper release retardant matrix composition for duloxetine HCl

The criteria for the selection of the polymer matrix formers was based on the fact of polymeric hydration to protect the tablet from rapid disintegration and dissolution and to extend the drug release rate. Various types of polymers and fillers were employed to form the matrix systems, e.g. Carbopol 71G-NF (carboxypolymethylene), a synthetic HMW acrylic acid polymer, pregelatinised starch (C-Pharm gel), guar gum, etc.

Studies were carried out using combination of Carbopol 71G-NF (granular grade of Carbomer) as the release retarding polymer in the matrix system along with Guar gum (GG1, GG2, GG4 and C3G2) were prepared. From the in-vitro study results it was inferred that the combination of Carbopol 71G-NF (30%) with guar gum (2%) (C3G2) is the most appropriate choice for the development of the desired matrix system but enteric coating is required to prevent initial drug release which is well known for their property to dissolve and release the contents of the dosage form on reaching the region where the pH is optimal for dissolution of the coat [15]. To retard the premature release of the drug in the upper segment of the GIT, HPMC-AS (3.0% w/v) was used at different coating

levels. Thus, Batch C3G2L1 was concluded to be the best formulation in the present study, because it prevented drug release in the stomach and further

provided sustained release to a sufficient extent i.e. it provided a uniform rate of drug release in the lower segment of the GIT.

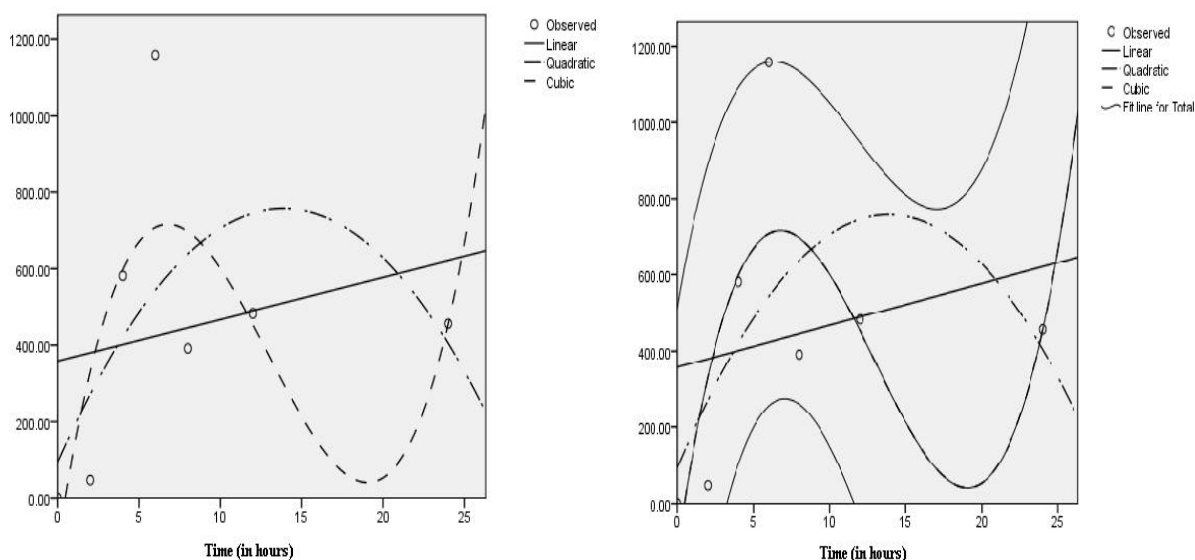


Figure 8. Curve fitting using least square method for determination correlation between mean plasma concentration (AUC_{0-24h}) against time at different intervals a) data fitting in different models b) confidence interval (at 95%) for fitting levels.

Rat forced swimming test (FST)

The pharmacodynamic performance of enteric coated sustained release tablets of duloxetine HCl of batch C3G2L1 was assessed with the help of well established rat forced swimming test (FST). The immobility period was recorded for each group at each time point. This immobilization represents a state of desperation in the rodents, which is a symptom seen in depression. Duloxetine HCl reduced the immobility time and thus resulted into increased activity, which is reflective of the efficacy of the drug in the present formulation. The data obtained was subjected to statistical analysis using Tukey's Test to compare the values of the control group to the other experimental groups.

Various mathematical models have been suggested for the depiction of drug release mechanism to check the goodness of fit, like zero order release kinetics, first order release kinetic, Higuchi's square root of time equation [16], Hixson-Crowell's cube root of time

equation [17] and Korsmeyer-Peppas power law equation [18-19]. The goodness of fit was evaluated by correlation coefficient values (R^2).

Table 5. Drug content of the Batch C3G2L1 after accelerated stability studies for 30 days (*Data represents mean \pm S.D)

Time points	Drug content (%) [*]
0 day	100.01 \pm 0.02
15 day	99.86 \pm 0.03
30 day	99.91 \pm 0.02

Thus, the mathematical model indicated that the drug release is mainly because of the change in surface area and diameter of the particles or tablets with time and

mainly applies in the case of systems that dissolve or erode over time.

From the pharmacokinetic data obtained, it was inferred that maximum therapeutic effect of the drug was observed within 6 h of administration and no drug release occurred in the initial 2 h which indicate the effectiveness of the enteric coat applied on the formulation.

Conclusions

Batch C3G2L1 was observed to be the most suitable for delaying the drug release for 2 h. It showed drug release of 2.24% at 2 h and 98.53% after 24 h. Stability studies were performed at 40°C/75% RH for 30 days indicating the batch C3G2L1 to be a stable formulation. In the pharmacodynamic model of depression (rat forced swimming test), the tablets were successful in decreasing the symptoms of depression. Further, pharmacokinetic data of batch C3G2L1 revealed that formulation gives t_{max} of 6 h and drug was found released even at the end of 24 h. Hence, the enteric-coated (HPMCAS) matrix composition consisting of Carbopol 71G NF as a 'release retarding agent' in combination guar gum, can be successfully used to protect the premature release of the hydrophilic drug moiety in the acidic environment of the GIT. The enteric coated tablets protected the drug degradation in the acidic environment.

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Conflicts of interest

The authors declare that they have no competing interests.

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